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Docket No.: 043956-0159

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

L.A. Johnson

Patent No.: 5,135,759

Issue Date: August 4, 1992

For: METHOD TO PRESELECT THE SEX OF OFFSPRING

SUBMISSION OF INTERIM EXTENSION OF PATENT TERM

Mail Stop Patent Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Submitted herewith is an application for extension of term of U.S. Patent No. 5,135,759 including:

Letter of Transmittal of Application for Interim Extension of Patent Term Under 37 C.F.R. § 1.790;

Contents of the application made by applicant and counsel pursuant to 37 CFR § 1.710 et seq. including Exhibits A, B, C and D; and

Letter in support of application from the exclusive licensee.

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Docket No.: 043956- 0159

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent 5,135,759
Patentee: L.A. Johnson
Issue Date: August 4, 1992

LETTER OF TRANSMITTAL OF APPLICATION FOR INTERIM EXTENSION OF PATENT TERM UNDER 37 C.F.R. § 1.790

Mail Stop Patent Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith is an application for extension of term of United States Patent 5,135,759 and a duplicate of the papers thereof.

The contents of the application consist of various statements made by the applicant and the undersigned counsel pursuant to 37 C.F.R. § 1.710 et seq. including EXHIBITS A, B, C, and D.

The Commissioner is hereby authorized to charge payment of any additional fees associated with this communication or credit any overpayment to Deposit Account No. 50-2134

A duplicate copy of this sheet is enclosed.

Respectfully submitted,

Gail E. Poulos

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1. **A COMPLETE IDENTIFICATION OF THE PRODUCT CURRENTLY UNDERGOING REGULATORY REVIEW:**

The product currently undergoing regulatory review is the medical device known as MicroSort® Sperm Separation Technology (hereinafter "the MicroSort system"), which enables an analysis of information from light emissions from stained sperm cells and separates the sperm cells into X- and Y-bearing populations based upon that analysis. The MicroSort system comprises: (1) a dye and reagents used for sperm specimen preparation and (2) a modified flow cytometer/cell sorter and laser used for sorting stained human sperm specimens into enriched populations of X- or Y- bearing sperm cells. The MicroSort system is an atypical medical device as this technology is intended for use by assisted reproduction facilities and is not intended to be manufactured and sold to customers as a stand alone medical device. The MicroSort system will have the following intended use statement in its labeling:

> The MicroSort® Sperm Separation Technology (MicroSort) is intended for use to separate X- and Y-bearing human sperm cells to improve the likelihood of conceiving an offspring of either gender for the prevention of sex-linked and sex-limited genetic disorders, or for family balancing.

The MicroSort system utilizes four principle processes to achieve its intended use:

(1) semen collection, evaluation and preparation, which are conducted prior to application of the MicroSort system;

(2) staining of sperm cells with a fluorochrome-containing, non-intercalating, vital DNA dye;

(3) sorting and collection of enriched populations of X- and Y-bearing sperm cells using a modified, flow cytometer/cell sorter equipped with a laser and multiple fluorescence detectors; and

(4) verification of the sorted sample for percentage of X- and Y-bearing sperm cells.

The MicroSort system is used to separate sperm after collection and initial evaluation of a sperm sample using standard procedures to remove debris and non-mobile sperm cells. After initial preparation a sperm sample is applied to the MicroSort system where it is first pretreated with a fluorescent non-cytotoxic dye, that selectively stains DNA, such as Hoechst bisbenzimide H 33342, under conditions that retain viability of the sperm cells and ensure that uniform staining takes place. For example, the staining conditions used in applying the MicroSort system include a temperature in the range of from about 30° to 39°C, preferably about 35°C for a period of time that retains viability of the sperm cells and ensures uniform staining, for example, about one hour at 35 °C.

The stained sperm sample is suspended in an electrically conductive and isotonic sheath fluid, injected into the sample injection port of the modified flow cytometer/sorter, which may have a beveled tip to assist in orienting the sperm cells, and carried into the flow cell. The flow cell is adapted to cause the sperm to flow single file and in proper orientation through a focused light source that has a wavelength range sufficient to excite the fluorochrome-containing dye. Excitation of the fluorochrome in the dye causes the DNA to which it is bound to fluoresce.

The fluorescence is captured by a combination of photon detectors, which are photomultiplier tubes that convert optical signals to proportional electronic signals for further electronic processing. The photon detectors are oriented at 0° and 90° relative to the light beam so as to enable a determination of properly oriented sperm *via* fluorescence magnitude and to control the second detector, which measures the

fluorescence intensity of only the properly oriented sperm cells. The second fluorescence measurement enables differentiation of X- and Y-bearing sperm cells. The sperm cells that are not properly oriented according to preset selection conditions can be eliminated before sorting, e.g., by an electronic gating system. The magnitude of fluorescence intensity of the emitted light from properly oriented sperm cells is quantified and analyzed to detect differences between X- and Y-bearing cells.

Once fluorescence differentiation between presumptive X- and Y-bearing cells is made, the sperm cells are sorted accordingly. The sample stream is first broken into uniform droplets by an ultrasonic transducer, causing each sperm cell to become encased in a liquid droplet. Droplets containing single sperm cells of the appropriate fluorescence intensity are given a charge and electrostatically deflected toward the opposite charged deflection plate of the cell sorter, enabling collection of X- and Y-bearing cells into separate containers.

2. **A COMPLETE IDENTIFICATION OF THE FEDERAL STATUTE UNDER WHICH THE REGULATORY REVIEW IS BEING CONDUCTED:**

The regulatory review is being conducted under Section 515 of the Federal Food, Drug and Cosmetic Act ("FFDCA"), 21 USC §301 et seq.

3. **A STATEMENT THAT THIS APPLICATION FOR INTERIM PATENT TERM EXTENSION IS BEING SUBMITTED WITHIN THE APPLICABLE SIX MONTH PERIOD AND IDENTIFICATION OF THE LAST DAY ON WHICH THE APPLICATION COULD BE SUBMITTED:**

This application is expected to be hand-delivered to the United States Patent and Trademark Office on June 5, 2009,which is within the period starting February 4, 2009,

six months before the original the patent is due to expire and ending on July 20, 2009, fifteen days before the original term of the patent is due to expire.

4. A COMPLETE IDENTIFICATION OF THE PATENT FOR WHICH AN EXTENSION IS BEING SOUGHT:

A complete identification of the patent is presented as follows:

Inventor:	Lawrence A. Johnson
Patent No.:	5,135,759
Issue Date:	August 4, 1992
Expiration Date:	August 4, 2009

5. A COPY OF THE PATENT FOR WHICH EXTENSION IS BEING SOUGHT

A copy of said patent is attached hereto as Exhibit A. The Patent has been assigned to the United States of America, as respresented by the Secretary of Agriculture. The assignment is attached hereto as Exhibit D.

6. A COPY OF ANY DISCLAIMER, CERTIFICATE OF CORRECTION, RECEIPT OF MAINTENANCE FEE PAYMENT, OR REEXAMINATION CERTIFICATE ISSUED IN THE PATENT

A copy of the maintenance fee statement of each of the three maintenance fees due at three and one half, seven and one half and eleven and one half years after grant and paid by USDA-ARS-OCI is attached hereto as Exhibit B. The statements have the payment dates of February2, 1996; February 4, 2000; and February 4, 2004, respectively.

No disclaimers, certificate of correction or reexamination certificate have been issued.

7. **A STATEMENT THAT THE PATENT CLAIMS A METHOD OF USING THE PRODUCT UNDERGOING REGULATORY APPROVAL REVIEW, AND A SHOWING THAT LISTS EACH APPLICABLE PATENT CLAIM AND DEMONSTRATES THE MANNER IN WHICH SUCH PATENT CLAIMS READ ON THE METHOD OF USING THE DEVICE**

U.S. patent 5,135,739 claims the use of the product for which regulatory approval is being sought. Claims 1, 5-20 and 23-26 of the patent claim methods to (1) sort intact, viable mammalian sperm into X- and Y-chromosome-bearing populations based on DNA content (claims 1-12 and 15-18), (2) preselect the sex of mammalian offspring (claims 13, 14 and 19) or (3) prepare sperm for cell sorting (claims 20 and 23-26). In each of these claimed methods the MicroSort system is used to sort mammalian, e.g., human, sperm cells into X- or Y-chromosome-bearing populations or is used, in part, to prepare human sperm for sorting on the basis of DNA content. Thus, claims 1, 5-20, and 23-26 claim methods of using the product (MicroSort system) for which regulatory approval is being sought.

Each of claims 1, and 5-20 and 23-26 is set out below:

1. A method for sorting intact, viable, mammalian sperm into X- and Y-chromosome-bearing populations based on DNA content, the method comprising:

a) staining intact, viable sperm collected from a male mammal with a fluorescent dye capable of selectively staining DNA in living cells by incubating the sperm with the dye at a temperature in the range of about 30°-39° C for a period of time sufficiently long for staining to take place uniformly but sufficiently short to preserve viability of the sperm;

b) passing the sperm into an electrically conductive and isotonic viability-supporting sheath fluid to form a suspension of sperm which are caused to flow singly in a stream of sheath fluid;

c) passing the sheath fluid containing the sperm before an excitation light source causing the stained DNA to fluoresce;

d) passing the sheath fluid containing the sperm through both a means for detecting the fluorescence of the stained DNA and also a cell sorting means, the means for detecting fluorescence having at least two detectors arranged such that a first detector determines the orientation of sperm on the basis of magnitude of fluorescence and controls a second detector to measure the DNA content of sperm on the basis of magnitude of fluorescence of those sperm that have been determined to be in a preselected orientation;

e) selecting by said cell sorting means the sperm having a DNA content corresponding to a desired chromosome which will produce a desired gender of offspring, and separating the selected sperm from nonselected sperm; and

f) collecting the selected sperm in a viability-supporting collecting fluid.

5. The method of claim 1, wherein said dye is bisbenzimide H33342 fluorochrome.

6. The method of claim 1, wherein said incubation is at a temperature of about 39° C for a period of about 1 hr.

7. The method of claim 1, wherein said incubation is at a temperature of about 35° C for a period of about 1 hr.

8. The method of claim 1, wherein said incubation is at a temperature of about 30° C for about 1.5 hr.

9. The method of claim 1, wherein said sheath fluid is phosphate-buffered saline solution, the solution also containing 0.1% bovine serum albumin to enhance sperm viability.

10. The method of claim 1, wherein said collecting fluid is modified test egg yolk extender.

11. The method of claim 1, wherein said sperm are hydrodynamically oriented in the flow of sheath fluid prior to being passed before said light source.

12. The method of claim 1, wherein said sperm are hydrodynamically oriented in the flow of sheath fluid by passing the fluid in a narrow stream through and out of a beveled injection tip prior to being passed before said light source.

13. A method to preselect the sex of mammalian offspring comprising:

a) sorting sperm according to the method of claim 1; and

b) inseminating a female mammal of the same species as the male mammal with the selected sperm in the collecting fluid.

14. A method to preselect the sex of mammalian offspring comprising:

a) sorting sperm according to the method of claim 1; and

b) fertilizing an egg obtained from a female mammal of the same species as the male mammal with the selected sperm in the collecting fluid.

15. The method of claim 1, further comprising eliminating sperm which are not properly oriented with an electronic gating system before sorting by said cell sorting means.

16. The method of claim 1, wherein the flow of sperm through the cell sorting means is regulated by an ultrasonic transducer.

17. The method of claim 1, wherein said sperm are sorted on the basis of X- or Y-chromosome DNA content with about 90% efficiency.

18. The method of claim 1, wherein said sperm are hydrodynamically oriented in the flow of sheath fluid and sperm which are not properly oriented are eliminated by an electronic gating system prior to being passed before said light source.

19. A method to preselect the sex of mammalian offspring comprising:

a) staining intact, viable sperm collected from a male mammal with a fluorescent dye capable of selectively staining DNA in living cells by incubating sperm with the dye at a temperature in the range of about 30.degree.-39.degree. C. for a period of time sufficiently long for staining to take place uniformly but sufficiently short to preserve viability of the sperm;

b) passing the sperm into an electrically conductive and isotonic viability-supporting sheath fluid to form a suspension of sperm which are caused to flow singly in a stream of sheath fluid;

c) passing the sheath fluid containing the sperm before an excitation light source causing the stained DNA to fluoresce;

d) passing the sheath fluid containing the sperm through both a means for detecting the fluorescence of the stained DNA and also a cell sorting means to measure the DNA content of the sperm on the basis of magnitude of fluorescence of the sperm;

e) selecting by said cell sorting means the sperm having a DNA content corresponding to a desired chromosome which will produce the desired gender of offspring, and separating the selected sperm from nonselected sperm; and

f) collecting the selected sperm in a viability-supporting collecting fluid.

20. A method for preparing intact, viable, mammalian sperm for sorting into X- and Y-chromosome-bearing populations based on DNA content, the method comprising staining intact, viable sperm collected from a male mammal with a fluorescent dye capable of selectively staining DNA in living cells by incubating the sperm with the dye at a temperature in the range of about 30° - 39 C for a period of time sufficiently long for staining to take place uniformly but sufficiently short to preserve viability of the sperm.

23. The method of claim 20, wherein said dye is bisbenzimide H33342 fluorochrome.

24. The method of claim 20, wherein said incubation is at a temperature of about 39° C. for a period of about 1 hr. 25.

25. The method of claim 20, wherein said incubation is at a temperature of about 35° C. for a period of about 1 hr.

26. The method of claim 21, wherein said incubation is at a temperature of about 30° C. for about 1.5 hr.

The following is a detailed discussion of how the claims listed above read on use of the MicroSort system:

The method of claim 1 comprises several steps, each of which relies on use of the MicroSort system. Claim 1 requires: (a) staining sperm collected from a mammal with a fluorescent, DNA-selective, vital stain; (b) suspending the stained sperm cells in an electrically conductive and isotonic viability-supporting sheath fluid; (c) causing the cells to flow in single file through an excitation light source, which causes the stained DNA to fluoresce; (d) detecting the fluorescence using multiple fluorescence detectors; (e) selecting cells on the basis of their DNA content *via* a cell sorter and separating selected sperm from non-selected sperm; and (f) collecting the selected sperm in a collecting fluid that supports sperm cell viability.

The fluorochrome-containing stain used in step (a) of claim 1 may be any DNA selective dye that is non-toxic to sperm, such as Hoechst bisbenzimide H 33342 fluorochrome [Col. 4, lines 27-29]. Step (b) of claim 1 requires use of an electrically conductive and isotonic sheath fluid to suspend the sperm cells after staining, which may be a phosphate

buffered saline solution containing. The isotonic sheath fluid may be supplemented with 0.1% bovine serum albumin to enhance sperm viability [Col. 4, lines 45-47]. Step (b) also recites that the stained sperm cells are caused to flow in single file. The flow may be controlled through use of a beveled sample injection tip which narrows the stream of flow to a single cell [Col. 3, lines 18-29]. In step (c) the sperm cells are passed before an excitation light source, which causes the stained DNA to fluoresce. The excitation light source may be a laser beam [Col. 3, lines 4-6], such as an Argon-ion laser that focuses ultraviolet light in the range of from 361 and 364 nm [Col. 6, lines 35-37], which is sufficient to cause the fluorochrome in the dye to fluoresce. The fluorescent light is collected by an optical lens assembly; and the signal transported to a photomultiplier tube, amplified, and analyzed by computer [Col. 3, lines 4-9]. Fluorescence is detected in step (d) through use of the MicroSort cell sorter having a combination of multiple detectors that are oriented relative to the light beam in such a manner as to enable determination of sperm cell orientation and measurement of DNA content of a properly oriented sperm cells. The detectors may be oriented at 0° and 90° relative to the light beam [Col. 3, lines 42-44]. Step (e) requires that the cell sorter selects sperm having the DNA content of a desired chromosome type (either X or Y). For sorting, the sample stream is broken into uniform droplets by an ultrasonic transducer [Col. 3, lines 44-46]. Droplets containing single sperm of the selected fluorescence intensity may be subjected to a charge and electrostatically deflected into a collecting vessel [Col. 3, lines 46-48] Finally, step (f) provides for collecting the selected sperm into a viability-supporting fluid. Collection fluid may be a modified test egg yolk extender containing a surfactant to enhance capacitation of the sperm prior to fertilization [Col. 4, lines 51-55].

It is evident from the discussion above that claim 1 reads on a method of using the MicroSort system, the product for which regulatory approval is being sought. Because each of claims 5-18 depend from claim 1 and include all of the limitations of claim 1, these claims also read directly on use of the MicroSort system.

Claim 5 claims a method according to claim 1 in which the dye used to stain the sperm is bisbenzimide H33342 fluorochrome, which is a fluorochrome-containing non-intercalating vital DNA dye. Claim 5 reads directly on the MicroSort system, which includes use of bisbenzimide H33342 fluorochrome to stain sperm cells prior to sorting. Claims 6, 7 and 8 specify the temperature and time at which the fluorescent dye is incubated with the sperm to effect staining. Claim 9 claims a method according to claim 1 wherein the isotonic viability supporting sheath fluid of step (b) is a phosphate-buffered saline containing 0.1% bovine serum albumin.

Claim 10 is a method according to claim 1 wherein the viability-supporting collecting fluid is modified test egg yolk extender.

Claim 11 is a method according to claim 1 wherein the sperm are hydrodynamically oriented prior to be passed through the light source of the cell sorter. Claim 11 reads directly on the use of the MicroSort flow cytometer/cell sorter system.

Claim 12 is a method according to claim 1 wherein hydrodynamic orientation of the sperm is effected by passing the sperm through a beveled injection tip prior to being passed through the light source. The modified flow cytometer/cell sorter of the MicroSort system may utilize a beveled sample injection tip to orient the sperm.

Claim 13 claims a method for preselecting the sex of mammalian offspring comprising the steps of (1) sorting sperm according to the method of claim 1 and (2) inseminating a

female member of the same mammalian species with the selected sperm in the collecting fluid.

Claim 14 claims a method for preselecting the sex of mammalian offspring comprising the steps of (1) sorting sperm according to the method of claim 1 and (2) fertilizing an egg of a female member of the same species with the selected sperm in the collecting fluid.

Claim 15 claims a method according to claim 1 wherein any improperly oriented sperm are eliminated with an electronic gating system prior to cell sorting. The modified flow cytometer/cell sorter of the MicroSort system can eliminate unoriented sperm by preset conditions, using, for example, an electronic gating prior to sorting.

Claim 16 claims a method according to claim 1 wherein the flow of sperm through the cell sorter is regulated by an ultrasonic transducer. The modified flow cytometer/cell sorter of the MicroSort system utilizes an ultrasonic transducer to break the sample stream into droplets.

Claim 17 claims a method according to claim 1 wherein cell sorting is effected on the basis of Y- or X- DNA content with about 90% efficiency. Claim 17 reads directly on the use of the MicroSort system.

Claim 18 claims a method according to claim 1 wherein sperm are hydrodynamically oriented in sheath fluid and improperly oriented sperm are eliminated by a gating system prior to being passed through the light source of the modified flow cytomer/cell sorter of the MicroSort system.

Claim 19 claims a method of preselecting the sex of a mammalian offspring comprising the steps (a) through (c), (e) and (f) as set forth in claim 1. Step (d) of claim 19 differs

from step (d) of claim 1 only in that the means for detecting fluorescence and the means for measuring DNA content are broadly defined in claim 19. Nonetheless, the method of claim 19 depends upon the use of the MicroSort system to effect sperm analysis, sorting and selection.

Claim 20 claims a method for preparing sperm for sorting into X- and Y-chromosome bearing populations based on their respective DNA content. The method of claim 19 includes the use of the staining process that is used in the MicroSort system. Thus, claim 20 reads directly on the use of the MicroSort system.

Claims 24-26 depend from claim 20 and further define the parameters for effecting staining to obtain viable, evenly stained sperm cells for sorting on the basis of DNA content. As such, claim 23-26 read directly on the use of the MicroSort system.

8. **A STATEMENT OF THE RELEVANT DATES AND INFORMATION PURSUANT TO 35 U.S.C. § 156(g) IN ORDER TO ENABLE THE SECRETARY OF HEALTH AND HUMAN SERVICES TO DETERMINE THE ELIGIBILTY OF THE PATENT FOR INTERIM PATENT TERM EXTENSION:**

Date of First Clinical Investigation:	1994
Date FDA requested GIVF submit an IDE:	Dec. 7, 1999
Date IDE filed:	April 24, 2000
IDE No.:	G000111
Date of Filing PMA Module 1 application:	March 31, 2008
The PMA (Module 1) Number:	M080005/M1
Date of Filing PMA Module 2:	July 22, 2008
The PMA (Module 2) Number:	M080005/M2
Date of Filing PMA Module 3:	March 31, 2009
PMA (Module 3) Number:	P090004

9. **A BRIEF DESCRIPTION OF THE SIGNIFICANT ACTIVITIES UNDERTAKEN BY THE MARKETING APPLICANT DURING THE APPLICABLE REGULATORY REVIEW PERIOD WITH RESPECT TO THE PRODUCT CURRENTLY UNDERGOING REGULATORY APPROVAL REVIEW AND THE SIGNIFICANT DATES APPLICABLE TO SUCH ACTIVITIES**

By virtue of a license agreement between the record owner of the subject patent, The United States of America as represented by the Secretary of Agriculture ("USDA"), and Genetics & IVF Institute (GIVF), the latter obtained certain rights under the subject patent. GIVF is licensed to obtain regulatory approval for and market the approved product in the United States. GIVF began its first clinical investigation of MicroSort for the prevention of sex-linked and sex-limited genetic disorders in 1993, after obtaining institutional review board (IRB) approval from the INOVA IRB at the Fairfax Hospital. In 1995, GIVF's own IRB approved the clinical study for sex-linked and sex-limited disorders, as well as family balancing. On December 7, 1999, the FDA requested that GIVF submit a significant risk IDE application for the ongoing clinical study of MicroSort. In compliance, GIVF submitted an IDE, which was approved on May 19, 2000. Since submitting the IDE, GIVF has provided the FDA yearly reports of an ongoing clinical study (annual IDE Progress Report).

GIVF submitted PMA application Module 1 on March 31, 2008 and subsequently an Amendment thereto on April 28, 2008. PMA application Module 2 was submitted July 22, 2008 and PMA Module 3 was submitted March 31, 2009. The marketing applicant (GIVF) believes that it has pursued its activities with due diligence throughout the regulatory review period, namely, the testing phase and the approval phase. Significant activities undertaken by GIVF with respect to the MicroSort system during the regulatory review period are briefly described as EXHIBIT C.

10. **A STATEMENT THAT IN THE OPINION OF THE APPLICANT THE PATENT IS ELIGIBLE FOR THE INTERIM EXTENSION**

Applicant believes that U.S. Patent No. 5,135,759 is eligible for interim patent term extension under 35 U.S.C. § 156(a) because it satisfies all of the requirements for such extension as follows:

(1) **35 U.S.C. § 156(a); 37 C.F.R. § 1.710**

U.S. Patent No. 5,135,759 claims a method of using a product (device)

(2) **35 U.S.C. § 156(a)(1)**

The term of the patent will not have expired before submission of this application.

(3) **35 U.S.C. § 156(a)(2)**

The term of U.S. Patent No. 5,135,759 has never been extended under 35 U.S.C. § 156(e)(1).

(4) **35 U.S.C. § 156(a)(3)**

This application for interim patent term extension is submitted by an attorney for the owner of record in accordance with the requirements of 35 U.S.C. § 156(d)(1)-(4) and rules of the U.S. patent and Trademark Office.

(5) **35 U.S.C. § 156(a)(4) [may not be necessary- see 35 U.S.C. § 156(d)(5)(A)(iii)]**

The MicroSort system, is currently the subject of a regulatory review and has not been commercially marketed or used as evident from Paragraph 9 above.

(6) **35 U.S.C. § 1.56(d)(5)(A)**

Applicant reasonably expects that the regulatory review period under 35 U.S.C. § 1.56(g)(3)(A) that began for the MicroSort system, which is the subject of U.S. Patent No. 5,135,759 may extend beyond the expiration of the patent term.

Applicant believes that the subject application is entitled to an interim patent term extension of up to one year or for a period that will terminate at the end of the 60-day period beginning on the day on which the MicroSort system receives permission for commercial marketing, whichever occurs first, as allowed by 35 U.S.C. § 156(e)(2).

11. **A STATEMENT THAT THE APPLICANT ACKNOWLEDGES A DUTY TO DISCLOSE INFORMATION MATERIAL TO ENTITLEMENT TO THE EXTENSION SOUGHT:**

Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services under 37 C.F.R. 1.765 any information that is material to the determination of entitlement to the extension sought herein.

12. **THE PRESCRIBED FEE FOR RECEIVING AND ACTING UPON THE APPLICATION FOR EXTENSION:**

Please charge the **Deposit Account 50-2134** (of USDA) in the amount of **$420.00** as the fee covering the instant application for interim patent term extension. The Commisioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to **Account No. 50-2134**

13. **THE NAME, ADDRESS AND TELEPHONE NUMBER OF THE PERSON TO WHOM INQUIRIES AND CORRESPONDENCE RELATING TO THE APPLICATION FOR INTERIM PATENT TERM EXTENSION ARE TO BE DIRECTED:**

Please forward all inquiries and correspondence relating to this application for interim patent term extension to:

Gail E. Poulos
USDA-ARS-OTT
5601 Sunnyside Avenue, Rm. 4-1183
Beltsville, Maryland 20705-5131
301-504-5302

14. **A DUPLICATE OF THE APPLICATION PAPERS, CERTIFIED AS SUCH:**

Four duplicate copies of these application papers, certified as such, are enclosed herewith. The undersigned patent attorney certifies under penalty of perjury that the attached duplicates of the application are true and correct copies of such papers.

DECLARATION OF ATTORNEY

I hereby declare that all statements made herein of my own knowledge are true; that all statements on information and belief are believed to be true; that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application; that I am a patent attorney authorized to practice before the United States Patent and Trademark Office; I am an authorized designee of USDA for the purpose of submitting this application for interim patent term extension, and hence, have the authority to submit and prosecute this application on behalf of the Secretary of Agriculture; and that I have reviewed and understand the contents of this application being submitted; that I believe the subject patent is subject to interim extension pursuant to 37 C.F.R. § 1.710; and that I believe that the subject patent meets the conditions for term extension as set forth in 37 C.F.R. § 1.790.

Respectfully submitted,

Gail E. Poulos
Reg. No. 36,327

Exhibit A

US005135759A

United States Patent [19]

Johnson

[11] Patent Number: 5,135,759

[45] Date of Patent: Aug. 4, 1992

[54] METHOD TO PRESELECT THE SEX OF OFFSPRING

[75] Inventor: Lawrence A. Johnson, Silver Spring, Md.

[73] Assignee: The United States of America as represented by the Secretary of Agriculture, Washington, D.C.

[21] Appl. No.: 692,958

[22] Filed: Apr. 26, 1991

Related U.S. Application Data

[63] Continuation of Ser. No. 349,669, May 10, 1989, abandoned.

[51] Int. Cl.[5] A61K 35/52

[52] U.S. Cl. 424/561; 436/63; 436/172; 435/2

[58] Field of Search 436/63, 172; 424/561

[56] References Cited

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Primary Examiner—Douglas W. Robinson
Assistant Examiner—Jean C. Witz
Attorney, Agent, or Firm—M. Howard Silverstein; John D. Fado; Curtis P. Ribando

[57] ABSTRACT

Intact X and Y chromosome-bearing sperm populations of rabbits and swine were separated according to DNA content using a flow cytometer/cell sorter. Sperm viability was maintained by special staining techniques and by sorting and collecting the sperm in nutrient media. The sorted sperm were surgically inseminated into the uteri of rabbits or swine. Of the offspring born from does inseminated with the sorted population of X-bearing sperm, 94% were females. Of offspring born from does inseminated with sorted Y-bearing sperm from the same ejaculate, 81% were males.

26 Claims, No Drawings

METHOD TO PRESELECT THE SEX OF OFFSPRING

This application is a continuation of application Ser. No. 07/349,669, filed May 10, 1989, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a method of preselecting the sex of offspring by sorting sperm into X and Y chromosome-bearing sperm based on differences in DNA content.

2. Description of the Prior Art

Gender of animal offspring is important to livestock producers. Because the dairy farmer has little use for most bull calves, the use of sexed semen to produce only females would make milk production more efficient. Swine farmers would produce pork more efficiently if they were able to market only female swine, because females grow faster than males.

In beef cattle and sheep breeds, the male grows at a faster rate than the female and hence is preferred for meat production.

In addition, the ability to specify male or female offspring should shorten the time required for genetic improvements, since desirable traits are often associated with one or the other parent. Planning the sex of cattle offspring is already practiced on a limited basis. This procedure consists of removing embryos from the cow, identifying their potential gender, and re-implanting only those of the desired gender. However, an ability to separate sperm into male-producing and female-producing groups before they are used for artificial insemination could enhance the overall value of offspring produced by embryo transfer.

Every living being has a set of paired chromosomes, which carry all the genetic material necessary to maintain life and also to propagate new life.

All but one pair of chromosomes are called autosomes and carry genes for all the characteristics of the body, such as skin, hair and eye color, mature size, and body characteristics. The remaining pair are called sex chromosomes. They carry the genetic material that specifies gender. One sex chromosome is called X, the other Y.

A sperm from the male or an egg from the female contains one of each pair of autosomes; in addition, in mammals the egg always contains an X chromosome, while the sperm always carries either an X or Y chromosome.

When a sperm and egg unite and the sperm carries the Y chromosome, the offspring is male (XY); however, if the sperm carries an X chromosome when it unites with the egg, the resulting offspring is female (XX).

The only established and measurable difference between X and Y sperm that is known and has been proved to be scientifically valid is their difference in deoxyribonucleic acid (DNA) content. The X chromosome is larger and contains slightly more DNA than does the Y chromosome. The difference in total DNA between X-bearing sperm and Y-bearing sperm is 3.4% in boar, 3.8% in bull, and 4.2% in ram sperm.

The amount of DNA in a sperm cell, as in most normal body cells, is stable. Therefore, the DNA content of individual sperm can be monitored and used to differentiate X- and Y-bearing sperm.

Since the difference in DNA mass in the sex chromosomes of most mammals is the only scientifically validated, measurable difference between X- and Y-bearing sperm, the chromosomal constitution [Moruzzi, J. Reprod. Fertil. 57: 319 (1979)] and/or measurement of DNA mass [Pinkel et al. (1), Science 218: 904 (1982); Pinkel et al. (2), Cytometry 3: 1 (1982); Johnson and Pinkel, Cytometry 7: 268 (1986); Johnson et al. (1), Gam. Res. 16: 1 (1987); Johnson et al. (2), Gam. Res. 17: 203 (1987)] are the only verifiable means other than fertility for determining the sex-producing capability of a population of sperm. The literature describes many physical, biochemical, and functional methods that have purportedly sexed sperm [Amann and Seidel, "Prospects for Sexing Mammalian Sperm," Colorado Assoc. Univ. Press, Boulder (1982)]; several of these methods have been tested for relative DNA content [Pinkel et al., J. Anim. Sci. 60: 1303 (1985); Johnson (1), Theriogenoloy 29: 265 (1988)]. However, no method has been proven in controlled experiments to actually affect the sex ratio of offspring.

Previous studies have demonstrated that the difference in DNA content between X and Y chromosome-bearing sperm can be repeatedly measured and the sperm sex ratio of a sample of semen predicted [Johnson and Pinkel, supra; Johnson et al. (1), supra; Johnson et al. (2), supra; Johnson (1), supra; Johnson (2), Cytometry, Suppl. 2: 66 (Abstract) (1988)]. Verifiable separation by sorting of X and Y sperm based on DNA content has been accomplished with the vole [Pinkel et al. (1), supra; Johnson, In "Beltsville Symposia in Agricultural Research X," P. C. Augustine, H. D. Danforth, & M. R. Bakst (eds.), Martinus Nijhoff, Boston, pp. 121–134 (1986)] and the chinchilla [(Johnson et al. (1), supra]. However, preparation procedures damaged DNA viability. The sorting of sperm nuclei from several mammalian (bull, boar, ram, vole, chincilla) species into separate X and Y chromosome-bearing populations at purities ranging from 92 to 99% has been accomplished [Johnson and Clarke, Gam. Res. 21: 335 (1988)]. Nuclear decondensation and pronuclear development was demonstrated in hamster eggs that had been microinjected with sorted X- or Y-bearing bull, boar, or ram sperm [Johnson and Clarke, supra].

SUMMARY OF THE INVENTION

It is an object of this invention to provide a method for sorting mammalian sperm into X and Y chromosome fractions based on DNA content.

It is a further object of this invention to teach a method of staining the DNA of mammalian sperm while maintaining viability of the sperm.

It is a further object of this invention to provide a sheath fluid adapted to be used in a cell-sorting apparatus while maintaining viability of sperm cells.

It is a further object of this invention to provide a collecting fluid capable of maintaining the viability of sorted sperm cells.

Other objects and advantages of this invention will become readily apparent from the ensuing description.

DETAILED DESCRIPTION OF THE INVENTION

I have now demonstrated the separation, by flow sorting, of intact, viable X and Y chromosome-bearing rabbit and swine sperm populations based on relative DNA content; surgical insemination of the sorted sperm into does; and the subsequent birth of sexed offspring

with a phenotypic sex ratio consistent with predictions based on the relative DNA content of the sorted sperm populations.

A flow cytometer measures the amount of fluorescent light given off when the sperm, previously treated with a fluorescent dye, pass through a laser beam. The dye binds to the DNA. The fluorescent light is collected by an optical lens assembly; the signal is transported to a photomultiple tube, amplied, and analyzed by computer. Because the X chromosome contains more DNA than the Y chromosome, the female sperm (X) takes up more dye and gives off more fluorescent light than the male sperm (Y).

For small differences in DNA to be detected between X and Y, the sperm must pass single file through the laser beam, which measures the DNA content of individual sperm.

In orthogonal flow cytometry, a suspension of single cells stained with a fluorochrome is made to flow in a narrow stream intersecting an excitation source (laser beam). As single cells pass through the beam, optical detectors collect the emitted light, convert the light to electrical signals, and the electrical signals are analyzed by a multichannel analyzer. The data are displayed as multi- or single-parameter histograms, using number of cells and fluorescence per cell as the coordinates.

In order to use an orthogonal flow cytrometric system to differentiate between X- and Y-bearing sperm DNA, a beveled sample injection tip and a second fluorescence detector in the forward position is required [Johnson and Pinkel, supra]. This paper is herein incorporated by reference. The modified system allows one to control the orientation of the flat ovoid sperm head as it passes the laser beam. Elimination of the unoriented sperm by electronic gating enhances precision. Typically, 80% of sperm nuclei (without tails) are properly oriented as they pass the laser beam.

In the modified Epics V flow cytometer/cell sorter, hydrodynamic forces exerted on the flat, ovoid mammalian sperm nuclei orient the nuclei in the plane of the sample stream as they exit the beveled injection tip. Fluorescent signals are collected simultaneously by 90 and 0 degree optical detectors, from the edge and flat side of the sperm nucleus, respectively. For sorting, the sample stream is broken into uniform droplets by an ultrasonic transducer. Droplets containing single sperm of the appropriate fluorescence intensity are given a charge and electrostatically deflected into collection vessels. The collected sperm nuclei then can be used for microinjection into eggs. Since the sperm nuclei have no tails, they cannot be used for normal insemination.

Accurate measurement of mammalian sperm DNA content using flow cytometry and cell sorting is difficult because the sperm nucleus is highly condensed and flat in shape, which makes stoichiometric staining difficult and causes stained nuclei to have a high index of refraction. These factors contribute to emission of fluorescence preferentially from the edge or thin plane of the sperm nucleus. In most flow cytometers and sorters, the direction of sample flow is orthogonal to the direction of propagation of the laser beam and the optical axes of the fluorescence detection. Consequently, fluorescence measurement is most accurate when the sperm fluorescence is excited and measured on an axis perpendicular to the plane of the sperm head [Pinkel et al. (2), supra]. At relatively low sample flow rates, hydrodynamics are used to orient tailless sperm so that DNA content can be measured precisly on 60 to 80% of the sperm passing in front of the laser beam. The modified Epics V system used in this study can measure the DNA content of tailless sperm from most species at the rate of 50 to 150 sperm per second [Johnson and Pinkel, supra].

Intact sperm (with tails), whether viable or nonviable, cannot be oriented as effectively as tailless sperm nuclei [Johnson (2), supra]. However, a 90-degree detector can be used to select the population of properly oriented intact sperm to be measured by the 0 degree detector. Since no hydrodynamic orientation is attempted, the sample flow rate can be much higher, which compensates somewhat for the fact that only 15 to 20% of intact sperm pass through the laser beam with proper orientation. In this invention, the overall flow rate was approximately 2500 intact sperm per second. The intact X- and Y-bearing sperm fractions were sorted simultaneously from the population of input sperm at a rate of 80–90 sperm of each type per second.

It is, of course, of critical importance to maintain high viability of the intact sperm during the sorting process and during storage after sorting but prior to insemination.

Of the factors involved in maintaining sperm viability, the method of staining, the sheath fluid, and the collecting fluid have been found to be especially important.

A nontoxic DNA stain must be selected. A preferred stain is Hoechst bisbenzimide H 33342 fluorochrome (Calbiochem-Behring Co., La Jolla, Calif.). To our knowledge, this fluorochrome is the only DNA binding dye that is nontoxic to sperm. Concentration of the fluorochrome must be minimal to avoid toxicity, and yet be sufficient to stain sperm uniformly and to detect the small differences in the DNA of X and Y sperm with minimal variation. A suitable concentration was found to be 5 μg/ml, but this may be varied from 4 to 5 μg/ml.

The sperm must be incubated with stain at sufficient temperature and time for staining to take place, but under mild enough conditions to preserve viability. Incubation for 1 hr at 35° C. was found to be acceptable, but ranges of 30° to 39° C. would also be effective. Incubation time has to be adjusted according to temperature; that is, 1.5 hr for 30° C.; 1 hr for 39° C.

Sheath fluid used in sorting cells must be electrically conductive and isotonic. A concentration of 10 mM phosphate buffered saline provided the necessary electrical properties, and 0.1% bovine serum albumin was added to enhance sperm viability by providing protein support for metabolism and viscosity for the sperm. The sheath fluid must be free of sugars and excess salts.

Dilution of sperm as occurs in sorting tends to reduce viability of the cells. To overcome this problem, sperm were collected in test egg yolk extender [Graham et al., J. Dairy Sci. 55: 372 (1972)] modified by adjusting the pH and adding a surfactant. Details of the composition of the extender are shown in Example 1. The surfactant is believed to enhance capacitation of the sperm prior to fertilization.

To confirm the DNA content and predict the sex of the offspring of surgically inseminated X or Y sorted sperm fractions, an aliquot of the sorted sperm was sonicated to remove the tails, stained, and the nuclei was reanalyzed for DNA content to predict the proportion of X and Y sperm.

Although the detailed description which follows uses the sorting of rabbit sperm as an example of this invention, it is expected that the sperm of most mammals could be effectively sorted by following these proce-

dures. Those skilled in the art will recognize that minor modifications may be made in the procedure without departing from the spirit and scope of the invention.

Rabbit semen was collected, diluted, and stained with a fluorochrome dye. Sperm were sorted in a modified Epics V flow cytometer/cell sorter.

After being sorted, sperm were surgically inseminated into the uteri of rabbits.

The results obtained by surgical insemination of does with sorted intact sperm are presented in Table I. Recovery of ova 40 hr post-insemination indicated that stained sorted sperm, as well as unstained unsorted sperm, were capable of fertilizing rabbit ova in vivo.

Inseminations were also made to determine the comparability of predicted sex of offspring to phenotypic sex. As the data in Table II indicate, the predictability of the phenotypic sex based on DNA analysis of the separated intact sperm was very high. Reanalysis of the sorted Y population used for insemination indicated that 81% of the sperm were Y-bearing. The sex ratio of offspring from these inseminations was identical to that predicted. These values were significantly different from theoretical 50:50 sex rates ($P<0.003$). Reanalysis of the sorted X-bearing sperm population used for insemination indicated that 86% were X-bearing and 14% were Y-bearing sperm. The phenotypic sex of the offspring from these inseminations was 94% female, which was different from the theoretical 50:50 ($P<0.0003$).

Inseminations were made with sorted X and Y populations that were recombined (recombined X and Y group) immediately before insemination. The assumption was made that the proportions of X and Y in the recombined samples were equal (50:50). The phenotypic sex resulting from the inseminations was 57% female and 43% male (Table II) and was not significantly different from the theoretical (50:50) sex ratio ($P=0.40$).

TABLE I

Fertilizing Capacity of Flow-Sorted Rabbit Spermatozoa After Intrauterine Insemination of Does

Treatment of Sperm	Number of Does Inseminated	Number of Ovulation Points	Number of Eggs Recovered	Number of Eggs Fertilized
Unsorted	2	16	9	9
Sorted	6*	59	46	39

*One doe accounted for 7 recovered and 7 unfertilized eggs.

TABLE II

Predicted and Actual Sex Ratios of Offspring After Intrauterine Insemination of Sorted X and Y Chromosome-Bearing Rabbit Sperm

Treatment of Sperm	Number of Does Inseminated	Number of Does Kindling	Total No. of Young Born	Percentage and Numbers of Offspring: Predicted % Males	Predicted % Females	Actual % Males (N)	Actual % Females (N)
Sorted Y	16	5	21	81	19	81 (17)	19 (4)
Sorted X	14	3	16	14	86	6 (1)	94 (15)
Recombined X and Y	17	5	14	50	50	43 (6)	57 (8)
Total	47	13	51	—	—	47 (24)	53 (27)

The phenotypic sex ratio of offspring born of does inseminated with either sorted X-bearing or sorted Y-bearing sperm was different ($P<0.0002$ for X and $P<0.001$ for Y) from the theoretical (50:50) sex ratio expected from untreated semen.

Embryonic mortality was significant in the does inseminated with sorted intact sperm. With a reasonably high fertilization rate (Table I), one would expect a kindling rate of near 80% and litter size of about six from does of this age and breed. However, the kindling rate across the three treatment groups averaged 28%, with an average litter size of 3.9. The cause of the apparent high rate of embryonic death is though to be due to the fluorochrome binding to the DNA and/or to the effect of the laser beam exciting the DNA bound fluorochrome. Earlier work has shown that sorted vole sperm nuclei that were microinjected into hamster eggs exhibited chromosome breakage in the developing sperm pronucleus [Libbus et al., Mut. Res. 182: 265 (1987)]. Those sperm had been sonicated, stained, sorted, and microinjected, a somewhat more rigorous treatment than the staining and sorting used in this study.

I have demonstrated that DNA can be used as a differentiating marker between X- and Y-bearing sperm, that DNA can be used to accurately predict the sex of offspring from separated X- and Y-bearing sperm populations, and that flow sorting is an effective means for separating viable X- and Y-bearing sperm populations suitable for production of offspring.

The following examples are intended only to further illustrate the invention and are not intended to limit the scope of the invention, which is defined by the claims.

EXAMPLE 1

Semen was collected from mixed breed mature bucks by use of an artificial vagina. Sperm concentration was determined with a hemocytometer. The semen was diluted with Tris buffer, pH 6.9, to a concentration of 10×10^6 per ml. Bisbenzimide H 33342 fluorochrome was added at a concentration of 5 μg/ml. The samples were incubated for 1 hr at 35° C. Intact sperm were sorted on a modified EPICS V flow cytometer/cell sorter. The stained intact sperm were excited in the ultraviolet (UV; 361 and 364 nm) lines of a 5-watt 90-5 Innova Argon-ion laser operating at 200 mW. Data were collected as 256-channel histograms. Sheath fluid was 10 mM phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA). Sperm were sorted into a test egg yolk extender.

The composition of the extender was N-tris(hydroxymethyl)-methyl-2-amino ethane sulfonic acid, 2.16 g; tris hydroxymethyl aminomethane, 0.51 g; dextrose, 0.1 g; streptomycin sulfate, 0.13 g; penicillin G, 0.08 g; egg yolk, 12.5 ml; Equex STM (Nova Chemical Sales, Scituato, Mass.), 0.5%; and distilled water, 50 ml. This mixture was centrifuged, and only the supernatant was used. The sorted sperm were concentrated by incubating at room temperature for 1 hr, after which the more dilute fraction was removed and the remainder was used for insemination 1 to 4 hr later.

18. The method of claim 1, wherein said sperm are hydrodynamically oriented in the flow of sheath fluid and sperm which are not properly oriented are eliminated by an electronic gating system prior to being passed before said light source.

19. A method to preselect the sex of mammalian offspring comprising:

a) staining intact, viable sperm collected from a male mammal with a fluorescent dye capable of selectively staining DNA in living cells by incubating sperm with the dye at a temperature in the range of about 30°–39° C. for a period of time sufficiently long for staining to take place uniformly but sufficiently short to preserve viability of the sperm;

b) passing the sperm into an electrically conductive and isotonic viability-supporting sheath fluid to form a suspension of sperm which are caused to flow singly in a stream of sheath fluid;

c) passing the sheath fluid containing the sperm before an excitation light source causing the stained DNA to fluoresce;

d) passing the sheath fluid containing the sperm through both a means for detecting the fluorescence of the stained DNA and also a cell sorting means to measure the DNA content of the sperm on the basis of magnitude of fluorescence of the sperm;

e) selecting by said cell sorting means the sperm having a DNA content corresponding to a desired chromosome which will produce the desired gender of offspring, and separating the selected sperm from nonselected sperm; and

f) collecting the selected sperm in a viability-supporting collecting fluid.

20. A method for preparing intact, viable, mammalian sperm for sorting into X- and Y-chromosome-bearing populations based on DNA content, the method comprising staining intact, viable sperm collected from a male mammal with a fluorescent dye capable of selectively staining DNA in living cells by incubating the sperm with the dye at a temperature in the range of about 30°–39° C. for a period of time sufficiently long for staining to take place uniformly but sufficiently short to preserve viability of the sperm.

21. The method of claim 20, wherein said mammal is a swine.

22. The method of claim 20, wherein said mammal is a bovine.

23. The method of claim 20, wherein said dye is bisbenzimide H33342 fluorochrome.

24. The method of claim 20, wherein said incubation is at a temperature of about 39° C. for a period of about 1 hr.

25. The method of claim 20, wherein said incubation is at a temperature of about 35° C. for a period of about 1 hr.

26. The method of claim 21, wherein said incubation is at a temperature of about 30° C. for about 1.5 hr.

* * * * *

Exhibit B

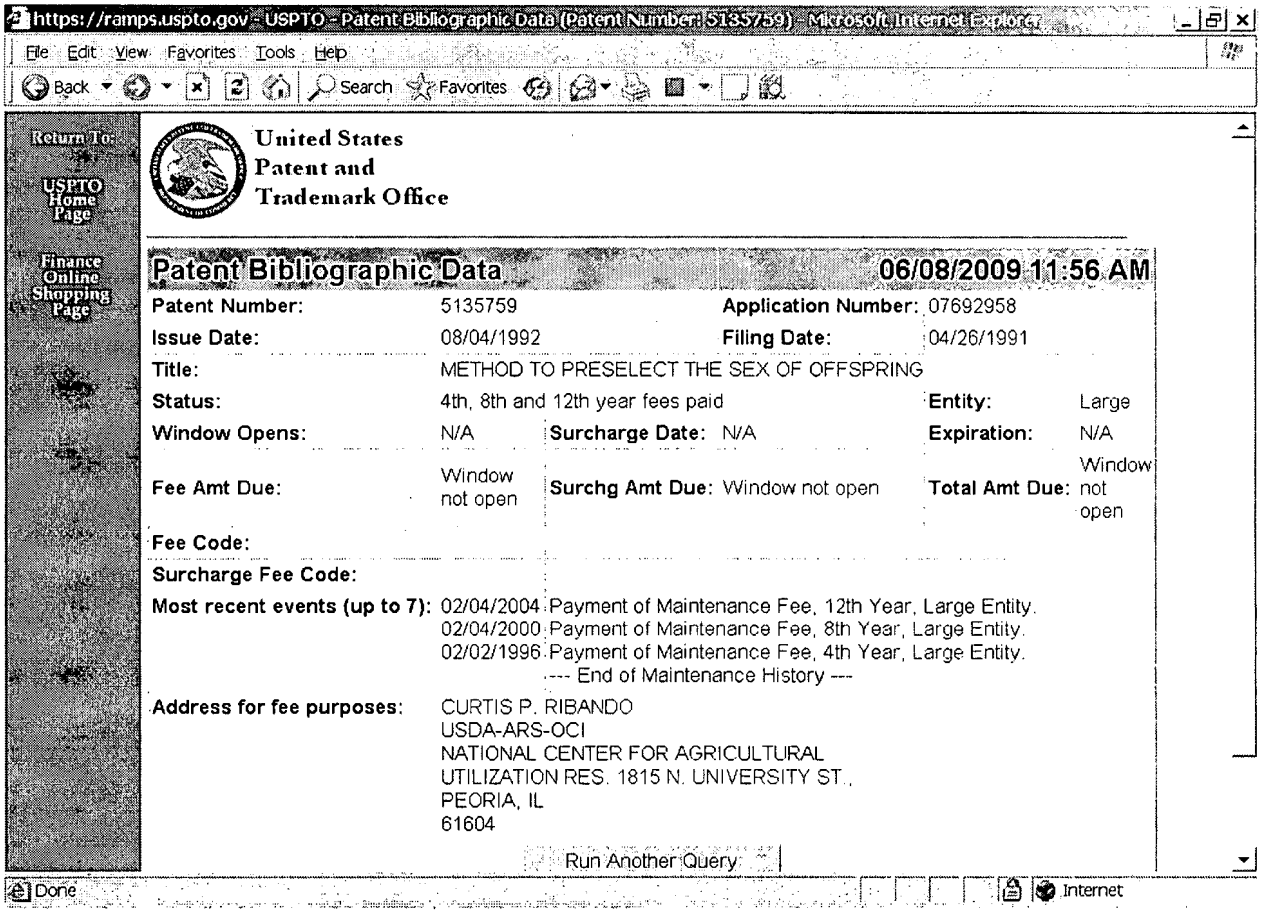
https://ramps.uspto.gov - USPTO - Patent Bibliographic Data (Patent Number: 5135759) - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back Search Favorites
Return To:
USPTO Home Page
Finance Online Shopping Page
United States
Patent and
Trademark Office
Patent Bibliographic Data
06/08/2009 11:56 AM
Patent Number: 5135759
Application Number: 07692958
Issue Date: 08/04/1992
Filing Date: 04/26/1991
Title: METHOD TO PRESELECT THE SEX OF OFFSPRING
Status: 4th, 8th and 12th year fees paid
Entity: Large
Window Opens: N/A
Surcharge Date: N/A
Expiration: N/A
Fee Amt Due: Window not open
Surchg Amt Due: Window not open
Total Amt Due: Window not open
Fee Code:
Surcharge Fee Code:
Most recent events (up to 7): 02/04/2004 Payment of Maintenance Fee, 12th Year, Large Entity.
02/04/2000 Payment of Maintenance Fee, 8th Year, Large Entity.
02/02/1996 Payment of Maintenance Fee, 4th Year, Large Entity.
--- End of Maintenance History ---
Address for fee purposes: CURTIS P. RIBANDO
USDA-ARS-OCI
NATIONAL CENTER FOR AGRICULTURAL
UTILIZATION RES. 1815 N. UNIVERSITY ST.,
PEORIA, IL
61604
Run Another Query
Done
Internet

Exhibit C-1

DATE	TO/FROM	SUBJECT
1993		Receipt of Institutional review Board (IRB) approval from the INOVA IRB at the Fairfax Hospital for clinical testing of MicroSort for the prevention of sex-linked and sex-limited genetic disorders.
1995		Receipt of IRB approval from GIVF's own IRB for the clinical study for sex-linked and sex-limited disorders, as well as family balancing.
12/7/99	FDA/GIVF	FDA requests that GIVF submit a significant risk IDE application for the ongoing clinical study of MicroSort.
12/30/99	GIVF/FDA	Outline of IDE faxed to FDA per request of Elisa Harvey and Colin Pollard (FDA).
1/5/00	GIVF/FDA	Conference call between FDA (Collin Pollard, Elisa Harvey, Diane Mitchell, Kike Kuchinski, Russ Owen) and GIVF (Ed Fugger, David Bick, Rekha Matkin, Barbara Lustig, Keith Blauer, Christine Brauer, Tom Tsakeris) to discuss outline for application.
1/14/00	FDA/GIVF	E-mail from Elisa Harvey (FDA) to Edward Fugger (GIVF) re IDE data requirements.
1/27/00	GIVF/FDA	GIVF submits a 513(g) request.
1/27/00	GIVF/FDA	GIVF submits response to FDA request for submission of IDE.
1/28/00	FDA/GIVF	CDRH assigns 513(g) submission number C000003.
1/31/00	FDA/GIVF	Telephone call from Wally Pellerite (FDA) to Bob Sheridan and Chris Brauer re 513(g) request. Discussion of custom device provisions and regulation of medicine.
2/22/00	GIVF/FDA	Supplement to 513(g) request submitted; information in response to Wally Pellerite's questions raised in telephone call of 1/31/00 provided.
4/18/00	FDA/GIVF	Letter from FDA: ODE determines MicroSort is a device requiring submission of IDE.
4/24/00	GIVF/FDA	GIVF submits IDE.

4/27/00	FDA/GIVF	FDA notification of document control number assigned to IDE (G000111)
5/4/00	GIVF/FDA	Facsimile to Elisa Harvey (FDA) with requested information on cytometer fluidics description and specification and outline of procedures for instrument disinfection validation.
5/19/00	FDA/GIVF	Letter to GIVF from Daniel Schultz (FDA) informing of conditional approval of IDE and citing deficiencies in the IDE; testing limited to a single site and 2500 subjects
6/12/00	GIVF/FDA	Facsimile to Elisa Harvey (FDA) re items to be discussed at 6/20/00 meeting of GIVF at FDA re IDE.
6/20/00	FDAGIVF	Meeting at FDA re conditional approval of IDE discussed
6/26/00	GIVF/FDA	Letter to Elisa Harvey (FDA) requesting extension of response period until July 17, 2000 to address deficiencies in IDE.
7/20/00	GIVF/FDA	Response to 5/19/00 deficiency letter submitted. Supplement 1.
8/17/00	FDA/GIVF	Receipt of letter granting conditional approval of IDE; testing limited to 1 site and 2500 subjects; 16 questions raised by FDA concerning dye safety, flow cytometric sporting, investigators, software, study protocol, case repot forms, and statistical plan.
10/9/00	GIVF/FDA	Submitted response to August 17, 2000 conditional approval letter; provided FDA Protocol 0001 (dated 10/9/00) Supplement 2).
11/10/00	FDA/GIVF	Letter of conditional approval of IDE; FDA raised additional 9 questions regarding dye safety, flow cytometric sorting, software, study protocol, and case report forms.
11/15/00	FDA/GIVF	Letter of conditional approval of IDE; several questions raised by FDA regarding dye safety, FISH probes, study protocol, software, and laboratory case report form.
11/30/00	GIVF/FDA	Letter to Colin Pollard (FDA) re delay in responding to FDA request for additional genotoxicity testing.

12/4/00	GIVF/FDA	Submitted response to FDA November 10, 2000 Conditional Approval Letter to address FDA questions. (Supplement 3).
12/19/00	GIVF/FDA	GIVF requests extension of period to respond to FDA November 15, 2000 letter.
12/21/00	FDA/GIVF	Conditional Approval of IDE; 4 questions raised by FDA concerning information requested in 11/10/00 letter from FDA, genotoxicity testing, and testing of non-human cells on clinical instrument.
1/2/01	FDA/GIVF	Telephone call from Elisa Harvey (FDA) granting extension to reply to November 15, 2000 letter.
1/22/01	GIVF/FDA	Submitted response to FDA November 15, 2000 Conditional Approval letter to address FDA questions (Supplement 4) regarding dye safety, FISH probes, study protocol, software, and laboratory case report form.
2/1/01	GIVF/FDA	Letter requesting extension of time to respond to FDA letter of December 21, 2000, raising 4 further questions information requested in 11/10/00 letter from FDA, genotoxicity testing, and testing of non-human cells on clinical.
2/5/00	GIVF/FDA	Facsimile sent requesting meeting re Supplement 2.
2/22/01	FDA/GIVF	Conditional Approval of IDE; 6 questions raised by FDA concerning dye safety, genotoxicity testing, software, and case report forms.
2/22/01	FDA/GIVF	Facsimile from Elisa Harvey (FDA) re compliance comments to change MicroSort promotional brochure and website.
2/26/01	GIVF/FDA	Submitted response to February 22, 2001 facsimile agreeing to include suggested changes.
3/1/01	FDA/GIVF	Conference call between Elisa Harvey (FDA) and Ed Fugger, K. Keyvanfar, Whit Athey (GIVF) to discuss questions concerning laser does applied to sperm.
3/8/01	FDA/GIVF	Conference call between E. Harvey, L. Magruder (FDA) and E. Fugger, K. Keyvanfar, C. Brauer (GIVF) to discuss Vysis DNA probes used in FISH.

3/12/01	GIVF/FDA	Request for extension of time to respond to December 21, 2000 and February 22, 2001 letters.
3/14/01	FDA/GIVF	Conference call between E. Harvey, R. Espiru (FDA) and C. Brauer, K. Keyvanfar, R. Fugger (GIVF) to discuss dye-DNA binding.
4/24/01	GIVF/FDA	Submitted response to questions raised in letters dated December 21, 2000 and February 21, 2001 re proposed genotoxicity testing and characterization of Hoechst 33342 stain. (Supplement 8).
5/25/01	FDA/GIVF	Conditional Approval Letter raising additional 4 questions. Regarding genotoxicity testing and dye-DNA binding.
5/31/01	GIVF/FDA	Submitted first annual IDE Progress Report. (Supplement 9).
6/29/01	FDA/GIVF	FDA requests additional information regarding Supplement 9; raises 9 questions regarding reporting results by sort indication (Family Balancing or Genetic Disease) malformations, preimplantation genetic diagnosis.
7/9/01	GIVF/FDA	Submitted response to May 25, 2001 questions. (Supplement 10).
8/7/01	FDA/GIVF	IDE approved: 1 site and 2500 subjects.
8/13/01	GIVF/FDA	Submitted response to FDA's June 29, 2001 request for additional information regarding 5/31/01 IDE Progress Report. (Supplement 11)
2/4/02	GIVF/FDA	Letter to FDA requesting additional testing site and permission to increase enrollment to 3500 subjects. (Supplement 17).
5/24/02	GIVF/FDA	Submitted second request for additional testing site and permission to increase enrollment to 3500 subjects. (Supplement 17).
5/30/02	GIVF/FDA	Submitted second annual IDE Progress Report. (Supplement 18).
6/27/02	FDA/GIVF	Letter from Nancy Brogdon approving request for second study site and increased enrollment.

7/5/02	GIVF/FDA	Telephone call between Sharon Lappalainen (FDA) and David Karabinus (GIVF) to clarify records keeping questions.
7/12/02	GIVF/FDA	Letter to Sharon Lappalainen (FDA) from David Karabinus (GIVF) regarding 7/5/02 telephone call and inviting FDA to meet with GIVF regarding detection of MicroSort-related genotypic effects.
6/13/03	GIVF/FDA	Submitted third annual IDE Progress Report.
7/21/03	FDA/GIVF	Letter raising 6 questions re 2003 Annual Progress Report regarding revising the informed consent, trisomy detection using PGD, dye binding assay protocol, and classification of malformations and patients within sort indications.
8/29/03	GIVF/FDA	Submitted response to July 21, 2003 letter to provide requested additional information for Annual Progress Report regarding revising the informed consent, trisomy detection using PGD, dye binding assay protocol, and classification of malformations and patients within sort indications.
8/29/03	GIVF/FDA	Submitted Protocol Amendment: Request to change Informed Consent Form. (Supplement 22).
9/16/03	FDA/GIVF	Email from Michael Bailey (CDRH) requesting additional information regarding malformations and revisions to the proposed dye binding assay.
9/24/03	GIVF/FDA	Submitted response to September 16, 2003 facsimile from Michael Bailey. (Supplement 23).
10/2/03	FDA/GIVF	Approval of Supplements 22 and 23 granted.
12/3/03	GIVF/FDA	Submitted Protocol Amendment: Request to add new sites and two BD flow cytometer devices to the study. Includes filing Protocol 0001 ver.3, dated December 3, 2003. (Supplement 24).
12/22/03	FDA/GIVF	Approval of Supplement 24 granted and expansion of clinical studies to 3 sites.
6/16/04	GIVF/FDA	Submitted fourth annual IDE Progress Report. (Supplement 25).

7/13/04	FDA/GIVF	Letter from FDA requesting further information regarding Annual IDE Progress Report concerning sort type and birth sex for karyoptype abnormalities, follow-up methods, outcome and other data presentation in annual reports, a typographical error, newly implemented media.
8/26/04	GIVF/FDA	Submitted response to July 13, 2004 letter FDA letter requesting additional information regarding sort type and birth sex for karyoptype abnormalities, follow-up methods, outcome and other data presentation in annual reports, a typographical error, and newly implemented media. (Supplement 26).
9/2/04	FDA/GIVF	Submitted Protocol Amendment: Response to July 13, 2004 letter providing results of verification and validation testing of human albumin and sperm preparation media used for sperm preparation plus information concerning the change in sperm preparation media and a change in MicroSort laboratory FISH procedures.
9/8/04	FDA/GIVF	Letter from Michael Bailey (CDRH) stating that Supplement 26 review is complete; no further questions raised.
10/1/04	FDA/GIVF	Letter requesting information concerning Annual IDE Progress Report and changes in sperm separation procedures.
11/12/04	GIVF/FDA	Submitted Protocol Amendment: Letter to FDA to answer FDA questions regarding proposed changes in sperm separation procedure and to provide answers to questions raised in 10/1/04 FDA letter. (Supplement 28).
12/14/04	FDA/GIVF	Approval of Supplement 28 granted.
12/27/04	GIVF/FDA	Report to FDA the resignation of Keith Blauer as Primary Clinical Investigator for MicroSort IDE.
1/24/05	GIVF/FDA	Report to FDA cancelation of plans to open and use a third testing site
2/15/05	FDA/GIVF	Acknowledgement of personnel change on MicroSort project and testing site changes.

6/17/05	GIVF/FDA	Submitted Protocol Amendment and data supplement: request to modify sperm comet assay protocol; reconsider GIVF's prior AMES studies; delay start of mouse lymphoma assay until test method determined; and provide information of stain binding assay. (Supplement 31)
8/30/05	GIVF/FDA	Submitted firth annual IDE Progress Report.
9/7/05	FDA/GIVF	Letter raising questions in regards to June 17, 2005 proposed protocol amendment.
9/28/05	FDA/GIVF	Letter raising questions in regards to Annual IDE Progress Report.
9/28/05	GIVF/FDA	Submitted Protocol Amendment: request increase in subject enrollment from 2500 to 3500 couples and increase in sample size from 750 to 1050 babies. (Supplement 33).
10/7/05	FDA/GIVF	E-mail from Michael Bailey requesting meeting. This meeting was formally requested by GIVF in the Meeting Request Packet submitted January 23, 2006 (see below).
10/25/05	FDA/GIVF	Approval of Supplement 33 granted.
11/10/05	GIVF/FDA	Submitted response to letter dated September 7, 2005 requesting further information regarding the dye not being a "Color Additive", modifications to the proposed Comet assay, request for reconsideration of the previously submitted Ames Assay results, sperm contacting materials, and reconsideration of mouse lymphoma assay test method.
11/14/05	GIVF/FDA	Submitted information requested in letter dated September 28, 2005 re Annual Report regarding changing format of fetal and birth sex results tables, determination of babies' sex, resolving discrepancies between 2004 and 2005 annual reports, and scheduling a meeting with FDA to discuss PMA submission.
11/16/05	FDA/GIVF	Email from Michael Bailey describing FDA suggestions regarding scheduling a meeting to discuss the MicroSort device. Included description of components of meeting request to include cover letter requesting meeting, a meeting agenda, a list of key questions GIVF would like FDA to address at the meeting, and a background package. FDA also included 7 questions it wanted addressed by GIVF.

11/29/05	FDA/GIVF	Email from Michael Bailey requesting composition of nine items which sperm contact during MicroSort processing and sorting.
1/23/06	GIVF/FDA	GIVF requests meeting based upon suggestion from FDA that GIVF should schedule meeting to discuss MicroSort device. GIVF submitted meeting request package following format suggested by FDA in November 16, 2005 email and attachment.
1/23/06	GIVF/FDA	IDE Interim Report, for the period January 1, 2005 through September 5, 2005 included with meeting request packet.
3/15/06	GIVF/FDA	Submitted information requested in November 29, 2005 letter from Michael Bailey regarding sperm contacting materials.
3/31/06	GIVF/FDA	Submitted information/agenda and GIVF attendees for proposed meeting with FDA.
4/6/06	GIVF/FDA	Submitted additional information requested in November 29, 2005 letter correcting the composition of the sample insertion port to clear Lexan polycarbonate, correction of the composition of the sample insertion rod to 316L stainless steel, and correction of the composition of the flow cell body to 316 stainless steel.
5/8/06	GIVF/FDA	Telephone call between Miriam Provost (FDA) and Jeffrey Gibbs (GIVF) to discuss reconsideration of de novo classification of the MicroSort device with special controls.
5/18/06	GIVF/FDA	Submitted sixth annual IDE Progress Report.
6/1/06	FDA/GIVF	Email from Michael Bailey (FDA) requesting reference reprint and requesting teleconference.
6/2/06	GIVF/FDA	Email FDA the article reprint requested 6/1/06 and respond to oral questions from FDA regarding malformations, sperm exposure to laser, baby follow-up, numbers of sorts, and consenting in telephone conference. Identify 11 questions from FDA to address.
6/2/06	FDA/GIVF	Email from Julia Corrado (FDA) to GIVF containing 6 questions regarding malformations.

6/2/06	FDA/GIVF	Email from Julia Corrado (FDA) containing 6 questions to GIVF regarding malformations as follow-up to telephone call.
6/6/06	FDA/GIVF	Send copies of current Informed Consent Document to FDA.
6/9/06	GIVF/FDA	Submit preliminary responses to FDA regarding the 11 questions posed by FDA in the 6/2/06 conference call.
6/15/06	FDA/GIVF	Email from Julia Corrado (FDA) containing proposed revisions to MicroSort Informed Consent Document.
7/11/06	GIVR/FDA	Email from GIVF to FDA containing GIVF revisions to Informed Consent Document revised by FDA 6/15/06.
7/19/06	GIVF/FDA	Submit synopsis of GIVF's responses to FDA's 6/2/06 and 6/6/06 questions, including reviews from each of 3 independent geneticists regarding their review of the cases identified by Dr. Corrado.
8/4/06	FDA/GIVF	Email from Julia Corrado containing a summary table of malformations from 5/18/06 annual report, requesting corrections and completion of missing information
8/23/06	GIVF/FDA	Email from GIVF to FDA containing completed table from FDA's 8/4/06 email.
9/8/06	FDA/GIVF	Email from Julia Corrado (FDA) containing proposed revisions to GIVF's revisions of Informed Consent Document.
11/17/06	GIVF/FDA	Email from GIVF to FDA containing GIVF revisions to Informed consent Document revised by FDA 9/8/06.
11/22/06	GIVF/FDA	Request for Pre-PMA meeting.
12/7/06	GIVF/FDA	Submitted Pre-PMA meeting submission package.
12/7/06	GIVF/FDA	Letter from Jeffrey Gibbs to Miriam Provost (FDA) re manufacturing section of IDE Annual Report.
1/24/07	FDA/GIVF	Email from Michael Bailey (FDA) containing proposed revisions to GIVF's 11/17/06 revisions of Informed Consent Document.

3/8/07	FDA/GIVF	Email from Michael Bailey stating that Julia Corrado had no further questions regarding the Informed Consent Document requested in the 6/6/06 email from FDA.
4/19/07	GIVF/FDA	Submitted Protocol Amendment: proposed clinical study changes. (Supplement 41).
4/26/07	GIVF/FDA	IDE Supplement submitted. (Supplement 42).
5/15/07	FDA/GIVF	E-mail from Michael Bailey (FDA) requesting withdrawal of certain information and re-submission as Pre-IDE.
5/16/07	FDA/GIVF	E-mail from Michael Bailey requesting information for Pre-IDE filing.
5/16/07	GIVF/FDA	Submitted Protocol Amendment: Withdrew sections for review in PMA as requested in Michael Bailey e-mail of May 15, 2007. (Supplement 43)
5/19/07	GIVF/FDA	Submitted seventh annual IDE Progress Report. (Supplement 44).
5/25/07	FDA/GIVF	Approved Supplements 41 and 43 re changes to clinical protocol and consent form.
6/18/07	FDA/GIVF	Letter requesting additional information and raising 5 questions re Annual IDE Progress Report, whether couples undergo more than one sorting procedure, formatting tables to reflect both the age of the egg source an the age of the embryo recipient in IVF/ICSI cycles, clarification of numbers of ectopic pregnancies, selective reductions, and elective terminations, identification of new aneuploidies, and whether karyotyping was performed in certain cases of malformations.

6/26/07	FDA/GIVF	Pre-PMA meeting with Miriam Provost ODE, Aron Yustein, MD ODE, Thinh Nguyen ODE, Nancy Brogdon ODE/DRARD, Karen Oliver ODE/DRARD, David A. Segerson ODE/DRARD, Herb Lerner ODE/DRAFD, Michael Bailey, Ph.D ODE/DRARD, Colin Pollard ODE/DRARD, Julia Corrado MD ODE/DRARD, Daya Ranamukhaarachchi, Ph.D. OIVD, Louise Magruder OIVD, Jason Schroeder, Ph.D. OSB, Richard Kotz OSB, Danica Marinac-Dabic, Ph.D., MD OSB, Nilsa Loyo-Berrios OSB, Paul Tilton IC, Sharon Murrain-Ellerbe (FDA) and David S. Karabinus, Ph.D., HCLD, Donald Marazzo MD, MPH, Harvey J. Stern, MD, Ph.D., Eugene R. Heyman, Ph.D., Brian C. Myhr, Ph.D., Jeffrey N. Gibbs, Carmelina G. Allis, Karen M. Becker, Ph.D., Kristin M. Zielinski (GIVF). Meeting objective to discuss data and information to be included in the PMA application. Items discussed were indications for use, manufacturing requirements (GMP/QSR), genotoxicity testing, clinical data presentation and statistical analysis. Also discussed were FDA administrative recommendations for PMA submission, possible postmarket studies, and PMA timing.
7/20/07	GIVF/FDA	Submitted response to June 18, 2007 letter requesting additional information regarding whether couples undergo more than one sorting procedure, formatting tables to reflect both the age of the egg source and the age of the embryo recipient in IVF/ICSI cycles, clarification of numbers of aneuploidies, and whether karyotyping was performed in certain cases of malformation. (Supplement 45).
10/4/07	FDA/GIVF	Comments from FDA regarding GIVF's "Pre-IDE" submission received.
3/31/08	GIVF/FDA	Filed PMA Module 1 with FDA.
4/10/08	GIVF/FDA	Requested continued access while the PMA Application is being prepared. (Supplement 46).
4/17/08	GIVF/FDA	Requested extension of time to submit annual IDE Progress Report until July 18, 2008. (Supplement 47).
4/28/08	GIVF/FDA	Submitted Amendment to PMA Module 1 to provide copies of references that were inadvertently omitted from PMA Module 1.
5/7/08	FDA/GIVF	E-mail re approval of request for extension to file Annual IDE Progress Report.

5/9/08	FDA/GIVF	Approval granted for Continued Access Study - limited to enrollment of 50 new patients/month for 6 months.
7/22/08	GIVF/FDA	Submitted PMA Module 2.
7/30/08	FDA/GIVF	Michael Bailey letter requesting updated information on clinical study.
8/11/08	FDA/GIVF	Clinical update requested by FDA.
8/12/08	GIVF/FDA	Submitted response to July 30, 2008 request for updated information on the progress of the clinical study. (Supplement 48).
8/13/08	FDA/GIVF	FDA requests additional information on the progress of the clinical study.
8/14/08	GIVF/FDA	Submitted response to FDA request for additional information on clinical update. (Supplement 49).
9/30/08	GIVF/FDA	Clarify questions regarding Michael Bailey (FDA, Colin Pollard, Julia Corado, Anne Lukas, Louise Magruder
10/3/08	GIVF/FDA	Submitted second continued access request t for expanded approval of GIVF's Continued Access Study with supporting additional information. (Supplement 50).
10/31/08	FDA/GIVF	Approval granted for Continued Access Study - limited to enrollment of 50 new patients/month for 6 months.
12/8/08	GIVF/FDA	Submitted dye purity testing protocol for FDA review. (Supplement 51).
12/30/08	GIVF/FDA	Submitted eight annual IDE Progress Report. (Supplement 52).
1/13/09	FDA/GIVF	FDA responds that dye purity testing protocol submitted 12/8/08 appears sufficient.
3/31/09	GIVF/FDA	Submitted PMA Module 3. PMA #P090004 assigned.
4/6/09	GIVF/FDA	Submitted third continued access request for expanded approval of GIVF's Continued Access Study with supporting additional information. (Supplement 53).
5/1/09	FDA/GIVF	Approval granted for Continued Access Study - limited to enrollment of 50 new patients/month for 6 months.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Exhibit D

TO: MERVIN E. BROKKE
USDA-ARS-NORTHERN REGIONAL RES. CTR.,
1815 NORTH UNIVERSITY STREET
PEORIA, IL 61604

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE U.S. PATENT AND TRADEMARK OFFICE ON THE REEL AND FRAME NUMBER REFERENCED BELOW. A DIGEST OF THE DOCUMENT HAS ALSO BEEN MADE AND APPEARS IN THE OFFICE'S RECORDS AS SHOWN:

ASSIGNOR: 001 JOHNSON, LAWRENCE A. DOC DATE: 04/17/89

RECORDATION DATE: 05/10/89 NUMBER OF PAGES 001 REEL/FRAME 5074/0550

DIGEST: ASSIGNMENT OF ASSIGNORS INTEREST

ASSIGNEE: 501 UNITED STATES OF AMERICA, THE, AS REPRESENTED BY THE SECR ETARY OF AGRICULTURE

SERIAL NUMBER 7-349669 FILING DATE 05/10/89
PATENT NUMBER ISSUE DATE 00/00/00

TITLE OF INVENTION: METHOD TO PRESELECT THE SEX OF OFFSPRING

INVENTOR: 001 JOHNSON, LAWRENCE A.

A S S I G N M E N T

WHEREAS, I (we), Lawrence Arthur Johnson

residing at 12516 O'Fallon Street, Silver Spring, MD 20904

having invented an improvement in "Method to Preselect the Sex of Offspring"

for which I (we) have made application for Letters Patent of the United States, executed concurrently herewith and further identified as Department of Agriculture Case No. 7008MB ; and

WHEREAS, the United States patent rights in said invention are assignable to the United States by virtue of my (our) having made the invention while in the employ of the United States Department of Agriculture under applicable law and regulations of the United States Department of Agriculture which render the patent rights so assignable; and

WHEREAS, the United States, as represented by the Secretary of Agriculture, is desirous of acquiring an assignment of said patent rights;

NOW, THEREFORE, in consideration of these premises, I (we) hereby assign said patent rights to the United States of America, as represented by the Secretary of Agriculture;

I (we) further grant to the Government of the United States a nonexclusive, irrevocable, royalty-free license in any patent which may issue on said invention in any foreign country, including the power to issue sublicenses for use in behalf of the Government and/or in furtherance of the foreign policies of the Government.

Still further, I (we) shall not employ such foreign patents to bar the sale or use, in any foreign country, of materials which are manufactured or otherwise produced essentially in the United States. Rather, I (we) shall grant patent licenses therein, at reasonable terms, to permit such sale or use; Provided, that I (we) shall not be compelled to grant such licenses in any foreign country where said materials are staple articles or commodities of commerce suitable for substantial use other than in infringement of such patents.

Lawrence A Johnson — 4/17/89
(signature) Lawrence Arthur Johnson (date)

(signature) (date)

(signature) (date)

RECORDED
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MAY 10 89
COMMISSIONER OF PATENTS
AND TRADEMARKS OFFICE

REEL 5074 FRAME 550

DOCKET NUMBER	ANTICIPATED CLASSIFICATION OF THIS APPLICATION: CLASS	SUBCLASS	PRIOR APPLICATION:
2108.91			EXAMINER J. C. Witz & D. W. Robinson

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on April 22, 1991
(Date of Deposit)

Curtis P. Ribando
Name of Applicant, assignee, or Registered Representative

Signature

April 22, 1991
Date of Signature

Address to:

Commissioner of Patents and Trademarks
Box FWC
Washington, D.C. 20231

This is a Request for filing a ☐ continuation-in-part ☒ continuation ☐ divisional application under 37 CFR 1.62 of prior application Serial No. 07/349,669, filed on May 10, 1989 entitled Method to Preselect the Sex of Offspring by the following named inventor(s).

FULL NAME OF INVENTOR	FAMILY NAME: Johnson	FIRST GIVEN NAME: Lawrence	SECOND GIVEN NAME: A.
RESIDENCE & CITIZENSHIP	CITY: Silver Spring	STATE OR FOREIGN COUNTRY: MD	COUNTRY OF CITIZENSHIP: U.S.
POST OFFICE ADDRESS	POST OFFICE ADDRESS: 12516 O'Fallon Street	CITY: Silver Spring	STATE & ZIP CODE/COUNTRY: MD 20904 U.S.
FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY

The above identified prior application in which no payment in the issue fee, abandoment of, or termination of proceedings has occurred, is hereby expressly abandoned as of the filing date of this new application. Please use all the contents of the prior application file wrapper, including the drawings, as part of this application. (note: 37 CFR 1.60 may be used for applications where the prior application is abandoned.)

1. ☐ Enter the amendment previously filed on _____ until entered in the prior application.
2. ☒ A preliminary amendment is enclosed.

cc:
OGC
Johnson
NRRC

ARS:NRRC:kam

The filing fee is calculated on the basis of the claims existing in the prior application as amended at 1 and 2 above.

Claims	(1) For	(2) Number filed	(3) Number extra	(4) Rate	(5) Calculations
	Total Claims	- 20=		X $ 20.00	$
	Independent Claims	- 3=		X $ 60.00	
	Multiple Dependent Claim(s) (if applicable)			+$ 200.00	
			Basic Fee		+ $630.00
			Total of above Caculations =		
	Reduction by 1/2 for filing by small entity (Note 37 CFR 1.9, 1.27, 1.28) if applicable. affidavit must be filed also.				-
			Total National Fee		$ 630.00

3. ☒ The Commissioner is hereby authorized to charge fees under 37 CFR 1.16 and 1.17 which may be required, or credit any overpaym to Deposit Account No. 01-0455 .

4. ☐ A check in the amount of $ _____ is enclosed.

5. ☐ A new oath or declaration is included since this application is a continuation-in-part which discloses and claims additional matter.

6. ☒ Amend the specification by inserting before the first line the sentence:

This application is a ☐ continuation-in-part, ☒ continuation, ☐ division, of application Serial No. 07/349,669, filed May 10, 1989.

7. ☐ A verified statement claiming small entity status is enclosed. (necessary even if a statement was filed in the prior application.)

8. ☐ Priority of application Serial No. _____ filed on _____ in _____ is claimed under 35 U.S.C. 119.

9. ☒ The prior application is assigned of record to The United States of America, as represented by the Secretary of Agriculture [Reel 5074-Frame 550; 5/10/89]

10. ☒ The power of attorney in the prior application is to: M. Howard Silverstein (22,699); John D. Fado (27,876); Curtis P. Ribando (27,976)

11. ☐ Also enclosed

Address all future communications to: (May only be completed by applicant, or attorney or agent of record)

Curtis P. Ribando
USDA-ARS-OCI
National Center for Agricultural
Utilization Research
1815 North University Street
Peoria, IL 61604

It is understood that secrecy under 35 U.S.C. 122 is hereby waived to the extent that if information or access is available to any one of the applications in the file wrapper of a 37 CFR 1.62 application, be it either this application or a prior application in the same file wrapper, the Patent and Trademark Office may provide similar information or access to all the other applications in the same file wrapper.

Sept 22, 1991
Date

Curtis P. Ribando
Curtis P. Ribando Signature

☐ inventor(s)
☐ assignee of complete interest
☒ attorney or agent of record
☐ filed under §1.34(a)

GENETICS & IVF
Institute

Celebrating 25 Years of Excellence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent 5,135,759
Patentee: L.A. Johnson
Issue Date: August 4, 1992

LETTER IN SUPPORT OF APPLICATION FOR INTERIM EXTENSION OF PATENT TERM UNDER 37 C.F.R. § 1.790

Mail Stop Patent Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Genetic & IVF Institute (GIVF) is the exclusive licensee of the above-referenced patent. GIVF is the also the marketing applicant before the Food and Drug Administration (FDA) for approval of use of the MicroSort cell sorter to sort human sperm, which is the subject matter of the claims of the above-referenced patent. GIVF has been the exclusive licensee of the subject patent throughout the entire regulatory review period. The United States of America as represented by the Secretary of Agriculture (USDA), the assignee (and licensor) of the patent and applicant for interim patent term extension of the patent is hereby authorized by GIVF to rely upon GIVF's activities before the FDA to obtain interim patent term extension of the subject patent.

Respectfully submitted,

David W. Wise

CEO Genetics & IVF Institute

June 2, 2009

3015 Williams Drive • Fairfax, Virginia 22031 • (703) 698-7355 • (800) 552-4363 • E-mail: givf@givf.com • www.givf.com